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### Pleasure from Food

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*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

2016

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Hoogeveen, H. R. (2016). *Pleasure from Food: Different perspectives on aging*. [Thesis fully internal (DIV), University of Groningen]. Rijksuniversiteit Groningen.

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# 4

# FUNCTIONAL SPECIALIZATION OF THE MALE INSULA DURING TASTE PERCEPTION

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*Published in Neuroimage (2015) doi:10.1016/j.neuro-  
image.2015.06.062*

# 4.1

## ABSTRACT

The primary gustatory area is located in the insular cortex. Although the insular cortex has been the topic of multiple parcellation studies, its functional specialization regarding taste processing received relatively little attention. Studies investigating the brain response to taste suggested that the insular cortex is involved in processing multiple characteristics of a taste stimulus, such as its quality, intensity and pleasantness. In the current functional magnetic resonance study, younger and older adult male subjects were exposed to four basic tastes in five increasing concentrations. We applied a data-driven analysis to obtain insular response maps, which showed that the insular cortex processes the presence of taste, its corresponding pleasantness, as well as its concentration. More specifically, the left and right insular cortices are differentially engaged in processing the aforementioned taste characteristics: representations of the presence of a taste stimulus as well as its corresponding pleasantness dominate in the left insular cortex, whereas taste concentration processing dominates in the right insular cortex. These results were similar across both age groups. Our results fit well within previous cytoarchitectural studies and show insular lateralization in processing different aspects of taste stimuli in men.

**Keywords:** cortical mapping, functional magnetic resonance imaging (fMRI), insula, lateralization, taste

## 4.2 INTRODUCTION

Information from the senses of vision, hearing, and touch is unimodally represented in distinct areas of the cerebral cortex, termed primary sensory areas. For taste, most researchers agree that the primary gustatory area resides in the insular cortex (see e.g., Small, (2010) for a review). The insular cortex is characterized by its widespread anatomical connections and its heterogeneous cytoarchitecture. To better understand the function of the insula, multiple studies have investigated the subdivision of the insular cortex based on its anatomical structure, functional connectivity and task-evoked activity. Cytoarchitecturally, the insula shows a smooth gradual change in its grey matter structure from agranular to granular in the anteroventral to posterodorsal direction (see e.g., Mesulam & Mufson, 1982). Correspondingly, diffusion weighted imaging studies have shown an anterior-posterior transition in white-matter connectivity variation within the insula (Cerliani et al., 2012; Nanetti, Cerliani, Gazzola, Renken, & Keysers, 2009). Studies investigating the functional connectivity of the insula have indicated that the anterior insula can be subdivided into two areas: the anterodorsal insula and anteroventral insula (Chang et al., 2013; Deen et al., 2011; Kelly et al., 2012). Kurth et al. (2010) investigated the task-evoked subdivision of the insula. Their large meta-study indicates that the insula functionally divides into areas associated with sensorimotor, cognitive, chemical sensory (i.e., olfactory and gustatory) and social-emotional domains. Furthermore, Kelly et al. (2012) found that results from clustering methods correspond remarkably well across the three different modalities (task-evoked co-activation, functional connectivity during rest and gray matter structural covariance), indicating strong agreement between anatomical and functional properties within the insular cortex.

Although the parcellation studies described above indicate that the insula divides into multiple subareas with distinct properties, the exact location of the primary gustatory area is still under debate. Experimental studies in non-human primates have suggested that this area is located in either the anteroventral or anterodorsal insula (Mesulam & Mufson, 1982; Yaxley, Rolls, & Sienkiewicz, 1990). However, a growing body of neuroimaging studies has indicated that the anteroventral insula processes taste in humans (Bender et al., 2009; Rudenga, Green, Nachtigal, & Small, 2010; Small, Zatorre, Dagher, Evans, & Jones-Gotman, 2001; Small, 2012). Meta-analyses of Kurth et al. (2010) and Veldhuizen et al. (2011) have shown that the anteroventral part of the insula is most associated with processing taste. Although Kurth et al. (2010) demonstrated right insula dominance for gustatory processing, Veldhuizen et al. (2011) did not find any proof of laterality.

There are several factors that complicate investigating the functional organization of the insula during taste perception. First, taste stimuli are always accompanied by somatosensory information. Therefore, brain activation may be evoked by somatosensory stimulation instead of taste or in addition to taste. To overcome this, several researchers contrasted the taste stimulus with a baseline stimulus, such as water or a tasteless solution containing artificial saliva. However, both water and tasteless artificial saliva still activate the primary gustatory area (De Araujo, Kringelbach, Rolls, & McGlone, 2003; Veldhuizen, Bender, Constable, & Small, 2007). Therefore, contrasting with such baseline stimuli reduces sensitivity.

A second complication resides in the fact that several subregions of the insular cortex have been reported to process different aspects of taste (e.g., pleasantness, intensity or presence). Selective attention to these different aspects seems to enhance brain activity in different parts of the insular cortex, although direct comparisons between these attention tasks have not yet been conclusive (Bender et al., 2009; Nitschke et al., 2006; Veldhuizen et al., 2007). Studies that tried to investigate the brain response while manipulating stimulus intensity, specifically, suggest that changes in intensity are associated with changes in activity in the middle insular cortex (Small et al., 2003; Spetter et al., 2010; Veldhuizen, Nachtigal, Teulings, Gitelman, & Small, 2010). Although many studies have focused on the orbital frontal cortex with respect to pleasantness, several have indicated that the insula also codes taste pleasantness (Bender et al., 2009; Cerf-Ducastel, Haase, & Murphy, 2012; Nitschke et al., 2006; Small et al., 2001). Since pleasantness and intensity highly correlate in many cases (Pfaffmann, 1980), it is hard to disambiguate the two, especially when both are not measured and/or manipulated within the same paradigm. Therefore, it is unclear whether the resulting insular responses represent either pleasantness or intensity coding.

Finally, a third complication stems from a methodological problem: researchers often used high taste concentrations because neuroimaging methods are rather insensitive to neuronal responses near detection threshold. Yet, high taste concentrations are often accompanied by disgust responses and may therefore elicit confounding mechanisms.

To investigate functional specialization of the insula during taste perception while trying to overcome the above-mentioned difficulties, we analyzed data from male subjects, who were exposed to basic tastes in increasing concentrations. We included both young and older adult males to obtain results on insular taste processing across age groups. For data analysis, we used a data-driven multivariate blind source separation approach that enabled us to disassociate insular activity related to multiple characteristics of the taste stimuli.

## 4.3 MATERIALS AND METHODS

### *Participants*

In this study, we acquired data of 21 healthy young males (mean age 23.9, SD = 2.81, range 19 – 30 years) and 19 healthy older males (mean age 65.8, SD=4.3, range 60 – 72). Participants were enrolled in the study on the basis of written informed consent. Participation was in accordance with the requirements of the medical ethical committee at the University Medical Center Groningen.

Participants were included when they reported no history of taste, smell, neurological, or psychological disorders. They were right handed, non-smoker for at least three months, and had normal or corrected to normal vision with MR-compatible lenses. Participants using any form of medication that possibly affected taste perception (i.e. gastrointestinal complaints, dry mouth, nausea, and taste disturbance) were not included in the study. Participants received a monetary compensation for participation.

One participant from the young male group was removed from the study after aborting the paradigm prematurely due to technical difficulties with the gustometer. Furthermore, one participant from the older males group was removed due to an unforeseen claustrophobic response.

Because food intake as well as brain responses to food images vary across the menstrual cycle (see e.g., Bryant et al. 2006; Frank et al., 2010; van Vugt, 2009), we anticipated that inclusion of female participants within the study would introduce extra undesired variation, negatively affecting the data-driven analysis. We therefore only included male participants.

### *Taste stimuli and delivery*

Stock solutions of sweet (560mM sucrose), salty (180mM NaCl), sour (10mM citric acid) and bitter (1mM quinine HCl) were created, matching taste stimuli used in previous studies (e.g., Bender et al., 2009; Jabbi et al., 2008; Rolls, 2011). These stock solutions were diluted with sterilized water to form series of 0%, 12.5%, 25%, 50% and 100% of the original stock concentrations. The 0% solution was also used for rinsing. Stimuli were delivered in the form of a 2ml bolus, using an in-house designed MR-compatible gustometer, consisting of 30 10ml syringes manually operated by an experimenter. Syringes were held firmly in place within the gustometer and five removable stops were placed between the plunger and barrel to ensure 2ml bolus deliveries. The syringes were attached to tubes (inner diameter 3mm; outer diameter: 4.1mm). Tubes containing water were connected together using stopcocks, such that only one tube ending provided a water stimulus. All tubes ended in a tight bundle of 17 tubes (one for water and 16 for tastants), which were held together in a central mouthpiece (a cut-off pacifier). The mouthpiece was secured to the headcoil and rested above the teeth of the participant, such that the participant was able to close his lips around the ending of the bundle (bundle diameter: ~14mm). The half-closed tubing system combined with the small tube diameter countered spontaneous leaking while at the same time impeding the participant to easily suck liquid from the tubes. Participants were instructed to try and keep their head as still as possible during tasting and swallowing. We did not specifically instruct them to limit tongue movement to minimize the risk of choking. Stimuli were administered manually by pushing the plunger to the next mechanical stop and administration lasted for approximately one second. Auditory countdown through headphones guaranteed timely stimulus administration by the experimenter.

### *Experimental design*

The experiment was divided into two sessions. In the first one-hour screening session, which was scheduled between 9.00 and 12.00 am, inclusion and exclusion criteria were checked, saliva samples were collected (results will be reported elsewhere), a hypogeusia-screening was performed using taste strips (Mueller et al., 2003; Steinbach et al., 2009), and participants were familiarized with the experimental procedure. The second session took place within seven days after the first session and contained a functional magnetic resonance imaging (fMRI) scan between 9.00 and 12.00 am or between 4.00 and 7.00 pm. Participants were instructed not to eat or drink during a two-hour period prior to the scanning session.

### *Hypogeusia screening*

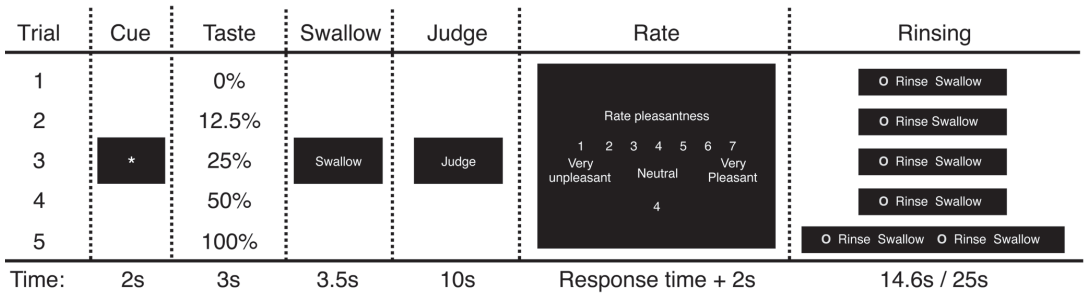
Taste function was assessed using spoon-shaped filter paper strips, which were impregnated with four basic tastes in four different concentrations (Mueller et al., 2003; Steinbach et al., 2009). Two tasteless strips were included. During each taste trial, participants were instructed to first rinse their mouth with water followed by placing a taste strip on the middle anterior third of the tongue. Subsequently, participants were instructed to identify the taste by choosing one out of five answers: sweet, sour, salty, bitter, and neutral (multiple forced choice). The order of the taste stimuli was randomized at each concentration, and stimulus presentation was in ascending (i.e., low to high) order of concentrations. The hypogeusia screening required approximately ten minutes. Identifying hypogeusia was based on the total number of correctly identified stimuli; participants scoring below 8 were excluded. We identified no hypogeusia in any of the recruited participants.

### *fMRI paradigm*

A schematic overview of the paradigm is given in Figure 1. Participants engaged in a tasting task containing 60 trials. During the course of the experiment, participants received visual cues and instructions in Dutch via a paradigm constructed in E-prime (Psychology Software Tools Inc., Pittsburgh). The paradigm was presented during four imaging runs. Each imaging run lasted for approximately 15 minutes (depending on reaction times) and was divided into 3 taste blocks. Each taste block contained a series of 5 trials with solutions of a single basic taste in ascending order of concentration. The start of every series was cued with the message “New Taste” (in Dutch: “Nieuwe Smaak”, duration: 2s). On a single-trial level, participants were warned for an upcoming taste delivery by an asterisk appearing centered on the screen (duration: 2s). Subsequently, 2ml of a basic taste was delivered in the mouth and participants were instructed to taste this stimulus with the cue “Taste” (in Dutch: “Proeven”, duration: 3s). After tasting, the participant was instructed to swallow the solution, cued as “Swallow” (in Dutch: “Slikken”, duration: 3.5s), followed by a period in which they needed to passively “Judge” the taste (in Dutch: “Beoordelen”, duration: 10s). Finally, a 7-point Likert scale appeared on the screen, ranging from “very unpleasant” to “very pleasant”. Participants were instructed to express their perceived pleasantness for the taste on the scale by using a button box held in their right hand. Every trial ended with a rinsing procedure, in which the participant received a 2ml bolus of sterilized water. At the end of every series of 5 trials, an extra rinsing procedure was included. The entire paradigm lasted for approximately 90 minutes, in which 264ml of liquid was consumed.

As baseline we included 4 periods of 15 seconds in each run, during which the participant was looking at a black screen with a red cross centred in the middle. The baseline periods were inserted at the start of every series of 5 trials and at the end of the run.

Although the number of repetitions per individual taste concentration is low (3 repetitions), we increased measurement sensitivity by 1) associating all stimulus presentations with pleasantness judgements, 2) integrating concentration information over multiple taste qualities (12 repetitions per concentration), 3) integrating taste quality information over multiple concentrations (12 repetitions per taste quality), and 4) optimizing the time resolution of the scanner paradigm (scan time per volume 0.852s) to increase sensitivity for BOLD signal detection (see below).



**Figure 1.** The figure shows an overview of the fMRI taste paradigm. The paradigm was divided into 4 runs containing 3 blocks each. Within each block every taste stimulus was delivered in a series of 5 trials containing increasing concentrations. Every trial started with a visual cue, followed by the taste. The participant was subsequently instructed to swallow, judge and provide a pleasantness rating for the stimulus. The first four trials ended with one rinsing procedure. After the fifth trial, the participant was instructed to rinse twice.

### Data acquisition

MRI scans were performed using a 3-Tesla MR scanner (Philips Intera, Best, the Netherlands) equipped with a 32-channel head coil.

A T1-weighted 3D fast field echo (FFE) whole brain image was obtained in transverse orientation for anatomical reference. Acquisition parameters: field of view (FOV)  $256 \times 232 \times 170\text{mm}$  (rl, ap, fh); voxel size 1mm isotropic; TR = 9ms; TE = 3.5ms; flip angle  $8^\circ$ ; SENSE factors: 2.5, 1 (ap, fh); 170 slices, scan duration = 246.3s.

Functional partial brain images were acquired in coronal orientation using the Principles of Echo-Shifting with a Train of Observations (PRESTO) sequence. Acquisition parameters: FOV  $230 \times 230 \times 81\text{mm}$  (rl, ap, fh); voxel size  $3.03 \times 3.59 \times 3\text{mm}$ ; matrix  $76 \times 64 \times 27$ ; TR = 20ms; TE = 30ms; flip angle  $7^\circ$ ; SENSE factors: 2.1, 1.9 (rl, ap); 27 slices, scan time per volume 0.852s. The coronal slices were centered on the brain stem ensuring the insulae were within the FOV.

In addition, 5 full brain PRESTO images were acquired with equal orientation and voxel size to the partial brain PRESTO images. The FOV was set to  $230 \times 230 \times 234\text{mm}$ ; 78 slices, scan time per volume 2.3s. The third full brain PRESTO image was used for an intermediate coregistration step between the partial brain PRESTO images and the anatomical image.

### Data preprocessing and analysis per individual

The functional data was analysed using SPM8 (Wellcome Trust Centre for Neuroimaging, <http://www.fil.ion.ucl.ac.uk/spm>) running in Matlab 2011b (The MathWorks Inc., Natick, MA). Functional images were registered to the mean functional image, co-registered to the third full brain PRESTO image and subsequently to the T1 image using the full brain PRESTO image as reference. The individual T1-weighted anatomical images were segmented into grey matter (GM), white matter (WM) and cerebral spinal fluid (CSF). By using Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL), a customized anatomical group template was created of all participants, which was subsequently normalized to the MNI template. This method optimises the inter-participant alignment (Ashburner, 2007). The images were smoothed with a 6mm full-width at half-maxi-



mum (FWHM) Gaussian kernel, resliced to a voxel size of 2x2x2mm and logarithmically transformed in order to express the signal measures in percent signal change (Langers & van Dijk, 2011).

For the statistical analysis per individual, we constructed mass-univariate general linear regression models in a block design because tastes stimulate taste receptors for several seconds. The regressors included: 1) conditions 'Taste', 'Swallow', 'Judge', and 'Rate' for each taste trial separately, allowing subsequent modeling of repetition effects at group-level; 2) global conditions 'New taste', 'Taste warning' (asterisk), and 'Rinse'; and 3) the realignment parameters and their first derivatives as covariates, correcting for head motion artefacts (Friston et al., 1996). In this way, the baseline represents the signal intensity during the period in which the participant was watching a red cross on the screen. The task-related regressors were convoluted with the canonical hemodynamic response function (HRF) and a high-pass filter of 128s was applied.

Due to technical difficulties, several PRESTO images were missing at random time intervals for 7 participants (on average 0.05% per data set). To minimize the effect of missing volumes, we replaced the volumes with the first PRESTO volume and included a separate regressor for each missing volume in the statistical analysis.

#### *Group-level data analysis*

##### **Region of Interest (ROI)**

To define a ROI, a mask of the insular cortex was created based on the Harvard-Oxford cortical and subcortical structural atlas, distributed with FSL v5.0 (The Oxford Centre for Functional MRI of the Brain, Nuffield Department of Clinical Neurosciences, University of Oxford, John Radcliffe Hospital, Oxford, OX3 9DU), comprising 4935 voxels (39.48 cm<sup>3</sup>).

##### **Group-level analysis**

First, we carried out a regular group-level analysis in SPM to show the average brain response to taste stimulus delivery versus baseline. This analysis served as a control step to ensure we successfully measured brain responses related to tasting. Resulting activation maps were thresholded at a global family-wise error (FWE) probability of  $P(\text{FWE}) < 0.05$ .

Subsequently, we applied blind source separation at the group-level. This method has been applied in previous studies to find both task-related brain activation patterns and intrinsic brain networks using Principal Component Analysis (PCA) (Langers & van Dijk, 2012), Factor Analysis (Langers, 2009) and Independent Component Analysis (Calhoun & Allen, 2013). Compared to classical mass-univariate analysis of fMRI data, this approach provides two major advantages: first, it avoids the obvious multiple-comparisons problem within mass-univariate analysis; second, by using blind source separation, the response dynamics (e.g., to taste concentration, quality or pleasantness) need not be exactly specified beforehand. For the group-level analysis in our study, we used factor analysis. Factor analysis was performed using PCA as preprocessing step. PCA is optimized for reducing the complexity of a data set by grouping as much signal variation as possible into as few components as possible, resulting in orthogonal components that may contain variation from multiple signal sources. Components can be unmixed by applying a factor analytic rotation

such as varimax, which attempts to form new components (factors), which better represent the latent variables that underlie the data. For fMRI data, the varimax rotation can be performed in either the spatial domain in which the contribution of each voxel will be regarded as a loading, or in the response profile domain in which the contribution of each condition will be regarded as a loading. When analyzing resting state data, the response profile domain is equivalent to a time-domain; in the current study it represents a taste condition domain. Because we intended to maximally separate pleasantness effects from concentration effects we chose to perform varimax in the taste condition domain.

The factor analysis was performed in R (version 3.1.2, 2014-10-31). For each participant (38 in total), 60 beta maps (4 basic tastes  $\times$  5 concentrations  $\times$  3 repetitions) of the condition 'Taste' were each flattened to a single column vector, insular cortex masked, and concatenated, thus creating a  $4935 \times 60$  insular response matrix  $Y$ . The mean response per voxel was then subtracted. Subsequently, all participant data were concatenated to obtain an aggregate  $4935 \times 2280$  matrix  $Y$ . For a succinct representation of the data, the matrix  $Y$  was decomposed into principal components using Singular Value Decomposition. The number of components to retain ( $nc = 2$ ) was based on Cattell's scree test (Cattell, 1966). Finally, the retained components were varimax rotated to form factors. These resulting factors each comprised an insular response map containing the amplitude variation of 4935 voxels (i.e., factor scores) and a corresponding response profile across all participants and all conditions, indicating how strongly the insular response map was represented in each condition per participant (i.e. factor loadings). The response profiles were constrained to unit root-mean-square amplitude, resulting in response profiles expressed in dimensionless arbitrary units and response maps expressed in percent signal change (see for more details: Langers and van Dijk, (2011) and Langers (2009)).

Note that all 0% concentrations constitute the same tasteless water stimulus. We included this water stimulus in the Factor Analysis to form a reference stimulus providing insight in how the insula responds to a gustatory stimulus without taste quality information.

### **Relating factor analysis results to taste stimulus characteristics**

In order to relate the obtained factors to taste quality, concentration and pleasantness, we used linear mixed models (LMM). LMMs are provided by the `lmer`-function in the `lme4` package for R (version 1.1-5, <http://cran.r-project.org/package=lme4>) (Bates et al., 2014; Pinheiro & Bates, 2000). Subsequent statistical tests on the LMMs were performed using Satterthwaite's approximation for the degrees of freedom, provided in the `lmerTest` package for R (version 2.0-11, <http://cran.r-project.org/package=lmerTest>) (Kuznetsova, Brockhoff, & Christensen, 2014). For all constructed models, the response profiles were entered as dependent variable. Taste quality, taste concentration, and perceived pleasantness were considered as independent variables in separate models. Finally, participants constituted a random variable. We performed likelihood ratio tests between nested model fits to test which independent variable best explained the variance expressed in the response profiles. When model comparisons are made, we will report the associated  $\chi^2$  statistic.

To test for response differences between both age groups, we additionally included the main and interaction effect of age group per model and calculated the mixed-effects repeated-measures ANOVA table based on Satterthwaite's approximation for the degrees of freedom (provided in the package lmerTest).

### **Behavioral (pleasantness) ratings**

To show we successfully manipulated pleasantness scores, effects of concentration and taste quality on perceived pleasantness are reported. For analyzing the pleasantness ratings, we also applied LMMs and calculated the mixed-effects repeated-measures ANOVA table. Here, pleasantness ratings were entered as dependent variable, while the taste quality and taste concentration constituted the independent variables.

## **4.4 RESULTS**

### *Behavioral results*

Figure 2 illustrates that we successfully manipulated pleasantness; the mixed-effect repeated-measures ANOVA table on pleasantness ratings indicates that pleasantness was different between taste qualities  $F(3, 2223) = 774.2, P < 0.001$  and between taste concentrations  $F(4, 2223) = 58.4, P < 0.001$ . Furthermore, the interaction between taste quality and taste concentration ( $F(12, 2223) = 58.5, P < 0.001$ ) indicates that changes in pleasantness induced by increasing concentration significantly differed between taste qualities.

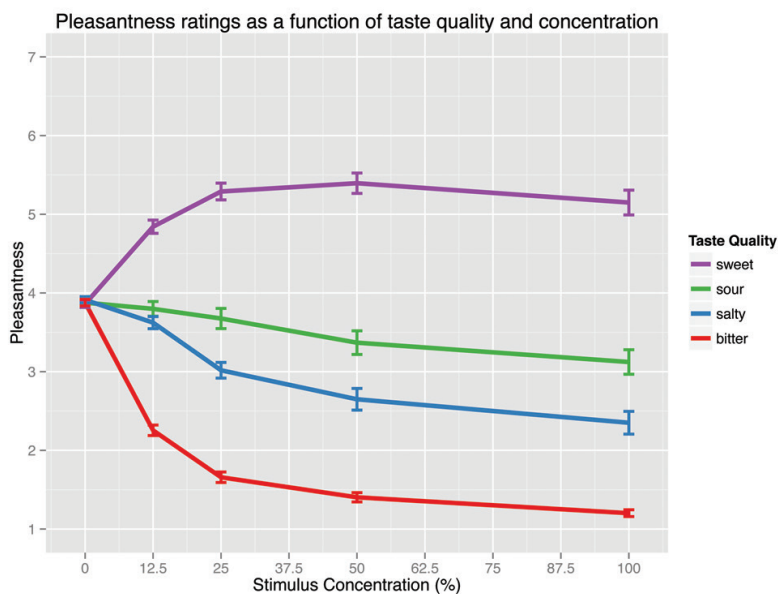
### *Mass univariate results on group-level*

Figure 3 and Table 1 show group-level activation results for the main effect of taste stimulus delivery ( $P(\text{FWE}) < 0.05$ , cluster size  $k > 100$  voxels). As expected, we found activation clusters in thalamic, sensory and motor areas as well as insular regions in response to all taste stimuli (see e.g., Veldhuizen et al., 2011). When masking for insular regions we found that mean activation peaked in the left anterior insula ( $T(37)=8.96, P(\text{FWE}) < 0.001$ ; MNI coordinates: -40,12,-6) and the right anterodorsal insula ( $T(37)=8.89, P(\text{FWE}) < 0.001$ ; MNI coordinates: 36,26,2).

### *Factor analysis results*

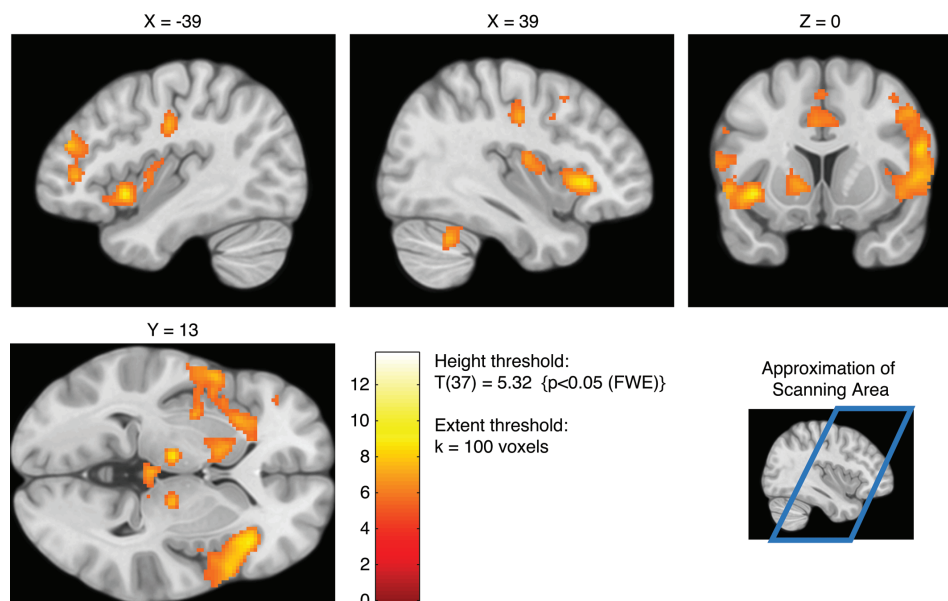
Following PCA, two components were retained explaining 43,7% of the total variance (32,6% and 11,2%, for PC1 and PC2, respectively), which were subsequently rotated using varimax. The resulting response maps of the factor analysis are given in Figure 4 while Figure 5 shows the corresponding response profiles as a function of taste quality, concentration and pleasantness.

The response map of the first factor was most pronounced in the left anteroventral insula extending towards the left middle insula and to a lesser extent within the right dorsal posterior insula. The relation with taste quality, concentration and pleasantness was threefold. First, LMM contrasts on the response profile indicated that the response profile loadings were higher during the presence of taste compared to water ( $T(2275) = 2.12, P < 0.05$ ), second, the response profile loadings were higher at



**Figure 2.** The figure shows the pleasantness ratings as a function of taste quality and concentration. Pleasantness ratings were measured 10 seconds after stimulus presentation during the fMRI paradigm. Error bars indicate the standard error of the mean (SEM).

#### Contrast: Liquid oral stimulus versus baseline



**Figure 3.** The figure shows the result of the SPM mass univariate group analysis. The result indicates the activated voxels in both insulae as a response to taste delivery in general. The color-coding of the intensity map is based on the T value generated by the contrast [all liquid stimuli - baseline]. The baseline consisted of looking at a red cross on a black screen. Therefore, the response pattern may include activity elicited by multiple characteristics of oral stimulus delivery (e.g., tactile, viscosity, taste and temperature information).

a low taste concentration compared to higher concentrations ( $T(2275) = 2.41$ ,  $P < 0.05$ ) as well as to water ( $T(2275) = 3.15$ ,  $P < 0.01$ ), and third, response profile loadings were negatively correlated with the perceived pleasantness of the taste ( $T(2278) = -2.26$ ,  $P < 0.05$ ). The response profile loadings did not differ between age groups as a function of taste quality ( $F(4,2270) = 0.44$ ,  $P = 0.78$ ), taste concentration ( $F(4,2270) = 2.09$ ,  $P = 0.08$ ) or pleasantness ( $F(1,2276) = 0.76$ ,  $P = 0.38$ ). Model comparisons indicated that a negative linear association between pleasantness ratings and the variance expressed in the response profile of the first factor significantly improved the model fit compared to the null-model ( $\chi^2(1) = 5.11$ ,  $P < 0.05$ ). Including a linear and/or quadratic effect of concentration did not significantly improve the model fit ( $\chi^2(1) = 0.002$ ,  $P = 0.97$  and  $\chi^2(2) = 0.27$ ,  $P = 0.87$  for the linear and quadratic effect, respectively). Furthermore, including the categorical variable taste quality did not improve the model fit over the null-model ( $\chi^2(4) = 7.59$ ,  $P = 0.11$ ). Therefore, the first factor is best characterized by a pleasantness effect.

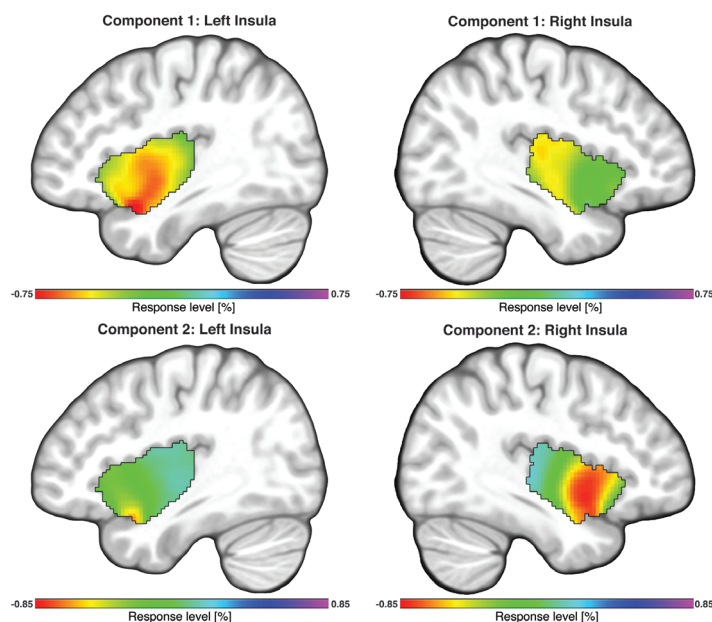
The response map of the second factor was associated with right anterior insula activity. LMMs indicated that the corresponding response profile is explained by a quadratic effect of stimulus concentration ( $T(2254) = 2.71$ ,  $P < 0.01$  and  $T(2109)$ ,  $P < 0.005$ , for the linear and the quadratic term, respectively). Thus, activity in the right anterior insula increased as a function of stimulus concentration up until the 50% stimulus concentration, after which the responsiveness diminished again. Again, we found no difference between age groups related to taste quality ( $F(4,2270) = 0.73$ ,  $P = 0.57$ ), taste concentration ( $F(3,2276) = 0.48$ ,  $P = 0.75$ ) or pleasantness ( $F(1,2276) = 0.029$ ,  $P = 0.87$ ) in the response profile of the second factor. Model comparisons indicated that a quadratic association between taste concentration and the variance expressed in the response profile of the second factor significantly improved the model fit compared to the null model ( $\chi^2(2) = 8.38$ ,  $P < 0.05$ ). Neither a linear effect of pleasantness ( $\chi^2(1) = 0.01$ ,  $P = 0.93$ ), a quadratic effect of pleasantness ( $\chi^2(2) = 0.18$ ,  $P = 0.91$ ), nor the categorical variable taste quality ( $\chi^2(4) = 3.95$ ,  $P = 0.41$ ) improved the model fit over the null-model. Thus, model comparisons indicated that the second factor is best characterized by a quadratic taste concentration effect.

## 4.5 DISCUSSION

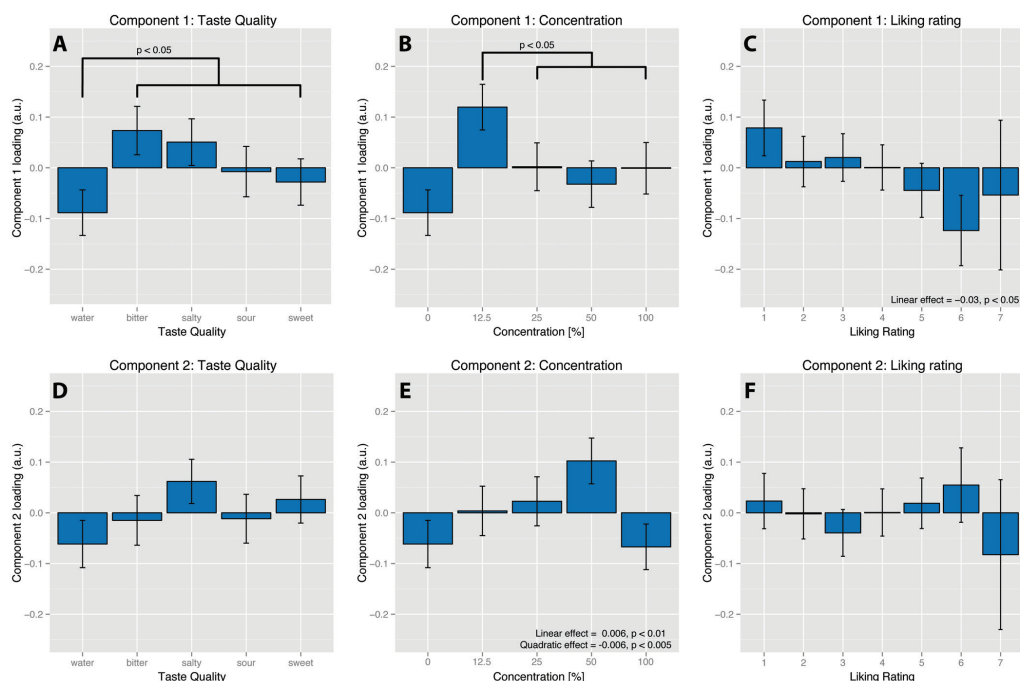
In the current study we investigated the functional specialization of the insula during the ingestion of basic tastes in both young and older adult males. Using factor analysis, we demonstrated that changes in BOLD signal evoked by three taste characteristics (i.e., quality, concentration, and pleasantness) could be decomposed into two factors, each containing a taste response map (i.e., factor scores) and an associated response profile (i.e., factor loadings). These response maps captured insular areas that showed similar behavior across all taste conditions and participants, while the associated response profiles indicate how strongly the group of insular areas within each response map was represented in each taste condition per participant. Our analysis indicated that the response map of the first factor was predominantly present in the left anteroventral to middle insula and captured an insular response to the presence of a taste stimulus as well as its corresponding pleasantness. Contrarily, the response map of the second factor mainly encompassed the right anterior insula. Analysis on the associating response profile indicated that the second factor was associated with taste concentration. Furthermore, we found that these effects were similar across young and older male participants.

Cluster number	Peak Region	Cluster size (cm <sup>3</sup> )	Peak voxel P <sub>(FWE)</sub>	T	MNI coordinates		
					x	y	z
1	Right Precentral Gyrus	35.20	< 0.001	13.71	62	-2	28
	Right Postcentral Gyrus		< 0.001	12.35	62	-10	20
	Right Postcentral Gyrus		< 0.001	12.27	60	-10	34
2	Left Postcentral Gyrus	26.28	< 0.001	11.95	-62	-8	22
	Left Anterior Insula		< 0.001	8.96	-40	12	-6
	Left Precentral Gyrus		< 0.001	8.04	-56	4	4
3	Left Amygdala	3.89	< 0.001	9.55	-22	2	-16
	Left Caudate		< 0.001	7.17	-12	10	-2
	Left Pallidum		0.003	6.44	-14	2	8
4	Left Thalamus	5.79	< 0.001	8.73	-10	-16	2
	Right Thalamus		< 0.001	8.55	16	-20	-6
	Right Thalamus		< 0.001	7.86	14	-16	4
5	Left Dorsolateral Prefrontal Cortex	3.56	< 0.001	8.22	-46	44	8
	Left Dorsolateral Prefrontal Cortex		< 0.001	8.11	-42	42	22
6	Paracingulate Gyrus	9.54	< 0.001	8.11	4	26	34
	Anterior Cingulate Gyrus		< 0.001	7.52	-6	32	22
	Supplementary Motor Cortex		0.001	6.97	4	2	56
7	Right Cerebellum	1.98	< 0.001	7.83	44	-54	-34
	Right Cerebellum		0.002	6.53	20	-44	-24
	Right Cerebellum		0.016	5.76	28	-52	-24
8	Left Cerebellum	1.84	< 0.001	7.48	-32	-54	-56
	Left Cerebellum		< 0.001	7.16	-30	-52	-34
	Left Cerebellum		< 0.005	6.24	-32	-50	-26

**Table 1.** Main effect of taste stimulus delivery.



**Figure 4.** The panels illustrate the Varimax rotated response maps expressed in percent signal change. Left insula maps correspond to  $X = -39$ , whereas right insula maps correspond to  $X = 39$  in MNI space. For component 1 the most extreme values are expressed in the left insula, whereas the right insula expresses the most extreme values for component 2.



**Figure 5.** The panels illustrate the response profiles for the first component (A-C) and the second component (D-F) as a function of taste quality (A,D), concentration (B,E) and pleasantness (C,F). Any significant statistical results from linear mixed models are indicated with the corresponding p-value. Panels A-C show that the results for the first component are threefold; the presence of taste scores significantly higher than no presence of taste (A), low concentrations score significantly higher than higher concentrations (B), and scores on the first response profile are negatively correlated with pleasantness (C). Panel E indicates that scores on the second response profile have a quadratic association with taste concentration. These scores did not significantly differ between taste qualities (D) or pleasantness ratings (F). Error bars indicate the standard error of the mean (SEM).



### *Results in the context of taste research*

Experimental studies in non-human primates have suggested that the primary gustatory area is located in either the anteroventral (Mesulam & Mufson, 1982) or anterodorsal insula (Yaxley et al., 1990). Although our group-level analysis indicated that the average response to a liquid stimulus is associated with i.a. the right anterodorsal insula, our factor analytic results are more pronounced towards the anteroventral insula. The latter is in line with meta-analyses on previous fMRI studies (Kurth, Zilles, et al., 2010; Veldhuizen, Albrecht, et al., 2011).

The first factor from our factor analysis encompassed the left anteroventral insula extending to the left middle insula and to a lesser extent the right dorsal posterior insula. Subsequent analysis showed that the first factor was associated with the presence of a taste. Interestingly, model comparisons showed that a linear effect of taste pleasantness best characterized the first factor. These results indicate that the associated areas not only process the presence of a taste, but also its corresponding valence. Although many studies have focused on the (orbital)frontal cortex regarding pleasantness (liking or valence) responses, several studies have indeed suggested an association between gustatory valence responses and left insula activity (Bender et al., 2009; Cerf-Ducastel et al., 2012; Frank et al., 2008).

The second factor was characterized by an association between the right anterior insula and stimulus concentration. Several studies have suggested that the right insula functionally relates to stimulus intensity (Small et al., 2003; Spetter et al., 2010). Although several authors also associated this region with taste pleasantness (Nitschke et al., 2006; Small et al., 2001), these studies were unable to rule out taste intensity effects, because taste concentration was not explicitly manipulated and/or subjective intensity ratings were not measured. We found no significant association between the second factor and pleasantness ratings. Model comparisons indicated that including pleasantness ratings did not improve a model fit compared to the null-model. Therefore, we conclude that activity within the right anterior insula is related to concentration and not to pleasantness. Interestingly, the responsiveness of the right anterior insula appeared to be quadratic, for which we have no definitive explanation.

With respect to age, Green et al. (2013) and Jacobson et al. (2010) reported offset differences between age groups in BOLD response to tastes within i.a. the insula. Here, we focused on the insular response variation across quality, concentration and pleasantness scores and found no differences between young and older participants. Therefore, we conclude that although there may be BOLD amplitude differences within the insula between young and older adults in response to taste, our results indicate that insular BOLD variation in response to multiple taste manipulations is similar across age.

### *Baseline shifts in BOLD signal during uncertainty*

The results from our analysis indicate that the first factor is more pronounced at a low stimulus concentration (see Figure 5b). This result may be explained by the findings of Bender et al. (2009). These authors showed that baseline activity shifts in the anteroventral insula during taste perception are associated with the participant's effort to detect and identify a taste. Therefore, the baseline shift observed in our results at low concentrations could be explained by selective attention towards the presence and/or identity of the taste.



### *Functional specialization*

Figure 6 illustrates that our results fit remarkably well with known insular cytoarchitecture and anatomy. The cytoarchitecture of the insula is characterized by a gradual change from agranular (Ia) to granular (Ig) in the anteroventral to posterodorsal direction (Cerliani et al., 2012; Mesulam & Mufson, 1982; Nanetti et al., 2009). Furthermore, Kelly et al. (2012) indicated that anatomical and functional insular parcellations largely overlap. In the current study we obtained a functional parcellation using taste stimuli. Our results show that, for taste perception, the main functional differences are also concentrated in anteroventral (agranular), anterior (dysgranular) and posterior (granular) insula. However, the functional specialization in taste perception is different between the left and right insular cortices.

For the first factor, we found that the left anteroventral (agranular) insula, left middle (posterior dysgranular) insula and right posterior (granular) insula were associated with processing the presence of a taste stimulus as well as its corresponding pleasantness. For the anteroventral insula, these findings fit into the general role of this area, as the anteroventral insula is involved in processing the emotional significance of environmental stimuli and production of affective states (Phillips, Drevets, Rauch, & Lane, 2003). Furthermore, the anteroventral insula is strongly connected to the orbitofrontal cortex, where final evaluation of reward and punishment of a stimulus is formed (Kringelbach & Rolls, 2004). For the left middle insula, activation was most pronounced within the posterior short gyrus (psg) and anterior long gyrus (alg). The right posterior insula activity was most pronounced in the posterior parts of the anterior long gyrus (alg) and posterior long gyrus (plg). Based on the cytoarchitecture of the posterior insula, the activation resides in the area Insula granular 2 (Ig2) (Kurth et al., 2010, figure 11 and Table 3). Meta-analyses clearly indicate a consistent involvement of the posterior insula during pain perception (Kurth, Zilles, et al., 2010; Lamm, Decety, & Singer, 2011). These findings are in line with connectivity studies that consistently show its relation with motor and somatosensory areas (Cauda et al., 2011). Thus, recruitment of the right posterior insula may indicate that perception of unpleasant tastes is associated with pain perception.

The second factor showed that stimulus concentration was most associated with right anterior (dysgranular) insula activity. Peak responses were most pronounced in the middle short gyrus (msg) and posterior short gyrus (psg).

Taken together, the two factors show that both the left and right middle insular cortices play a role in taste perception. The middle (dysgranular) insula is cytoarchitecturally regarded as the transition area between the anteroventral (agranular) and posterior (granular) insula (Cerliani et al., 2012; Mesulam & Mufson, 1982; Nanetti et al., 2009). Furthermore, intracranial recordings have shown that the middle insula is functionally highly connected with both the anterior and posterior insula (Almashaikhi et al., 2013). As the emotional significance (anteroventral insula) and the potential harmfulness/painfulness (posterior insula) are important during the evaluation of a food stimulus, the middle insula might be a suitable region to process taste stimulus properties.

### *Lateralization*

Faurion et al. (1999) attributed the insular lateralization of taste perception to handedness. According to their study the left insula predominantly responded to taste in right-handed participants, while this result was reversed for left-handed participants. Although our results are consistent with this finding, as all participants were right handed and the left insula predominantly responded to the presence of a taste, previous studies have shown inconsistencies (Small, Zald, Jones-Gotman, et al., 1999; Veldhuizen, Albrecht, et al., 2011).

An alternative explanation for this laterality was given by Small et al. (1999). They hypothesized that gustation is dominated by the right insula as a result of a left insular dominance in language processing. Although we can neither confirm nor refute these hypotheses, our results demonstrate that the left and right insula are differently involved in processing the taste stimulus. This may explain lateralized findings in previous studies in which pleasantness and intensity were not jointly measured.

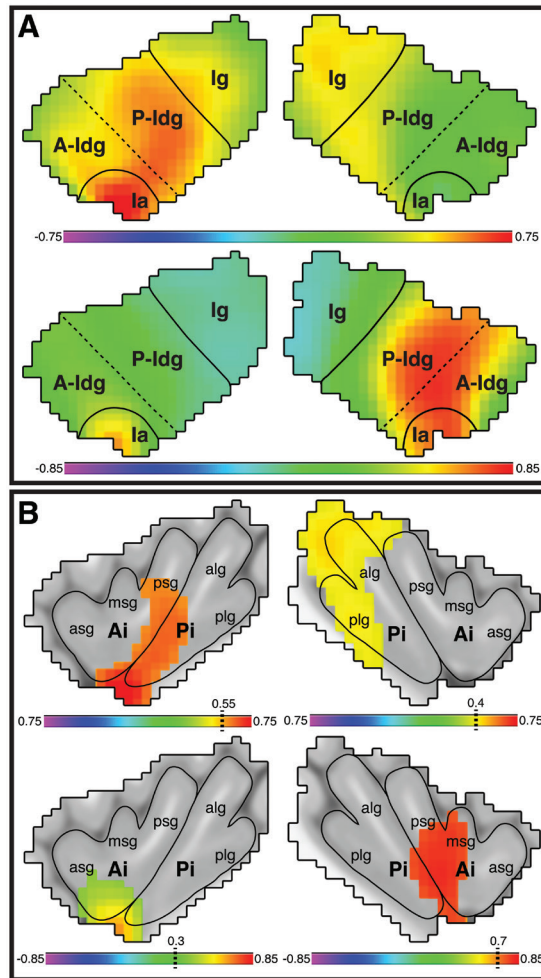
Interestingly, our finding that the left insula dominates in processing pleasantness is in agreement with a meta-analysis on the lateralization of affective processing in the insula by Duerden et al. (2013). The authors included 143 emotion studies and concluded that: “males processed emotional stimuli predominantly in the left anterior/mid-insula and the right posterior insula”. This constitutes a finding that very closely matches the pleasantness results presented in the current study.

### *Limitations*

A possible alternative interpretation of the negative association between the first factor and pleasantness ratings is that this factor is associated with aversiveness coding. However, as we only measured pleasantness ratings, the factor was interpreted in terms of pleasantness. We believe that further experiments are needed, optimized on covering the full pleasantness and aversiveness range per participant, in order to investigate differences in aversiveness and pleasantness processing in the insula. These experiments should also shed more light on the discussion whether pleasure and aversiveness are part of the same neurobiological continuum. We believe that this discussion does not have a definitive answer yet (also see Kringelbach and Berridge (2010), pages 12-14).

To minimize carry-over effects between taste qualities, we presented stimuli in ascending concentrations. By doing so, we might have introduced anticipatory effects causing baseline shifts in BOLD signal at low taste concentrations as shown in Figure 5b. Although, a fully randomized design may have reduced these anticipatory effects, low taste concentrations would still require more selective attention for taste quality identification.

The perceived pleasantness of an individual taste stimulus varies widely between consumers (Dalenberg, Nanetti, Renken, de Wijk, & Ter Horst, 2014; Rudenga & Small, 2013; van den Bosch et al., 2014). Although a sweet taste is widely considered as a positive stimulus, groups of sweet likers and sweet dislikers exist, who show differential brain responses to the same sweet stimulus (Rudenga & Small, 2013). Therefore, stimulus quality is a poor indicator for affective value. In the current study, we took account of this by measuring perceived pleasantness, providing us with personalized affective scores. By doing so, we directed the attention of our participants to the



**Figure 6.** Panel A illustrates the response maps projected on an approximation of the cytoarchitectural diagram as described by Mesulam & Mufson, (1982). The panel shows how the response maps distribute the agranular insula (Ia), anterior dysgranular insula (A-I dg), posterior dysgranular insula (P-I dg) and granular insula (Ig). Panel B illustrates how peak responses in the response maps are associated with the anterior short gyrus (asg), middle short gyrus (msg) and posterior short gyrus in the anterior insula (Ai); and the anterior long gyrus (alg) and posterior long gyrus (plg) in the posterior insula (Pi). The response maps are identical to panel A, but have been thresholded for visualization purposes; thresholds are depicted on the intensity bars, which indicate percent signal change with respect to the mean response.

pleasantness of the taste. Attention to a specific stimulus property is reflected in baseline activation shifts within associated brain areas. For taste, several studies have indicated that attention to a particular taste attribute, such as its intensity or pleasantness, enhances activity in specific insular areas (Bender et al., 2009; Nitschke et al., 2006; Veldhuizen et al., 2007). This finding may indicate that the rating-task in our paradigm poses a confounding factor.

Our results are limited to the male population. Cornier et al. 2015 and Haase et al. (2011) showed that differences exist in gustatory processing between males and females. Therefore, it remains unclear whether similar lateralized effects exist in insular gustatory processing within females. This question should be addressed in future studies.

In this study, we did not find evidence for differences between young and older males. However, since our results were limited to the insular cortex, the processing of taste may still differ between young and older individuals when additional brain areas are considered.

### *Conclusion*

Our study is the first to investigate the functional specialization of the insula during processing of basic tastes. In accordance with previous studies, we show that the bilateral insula of males not only processes the presence of taste, but also its pleasantness and concentration. Our analysis indicates that these taste characteristics associate with two topographical maps within the bilateral insula. Moreover, we show that the left and right insula are differently engaged in processing taste presence, pleasantness and intensity. Processing the presence of taste as well as its corresponding pleasantness is dominant in the left anteroventral insula and to a lesser extent in the right posterior insula, whereas taste intensity processing is associated with right anterior insular activity. These results were similar across both age groups that were included in this study. Furthermore, we show that our results fit well within previous task-related and cytoarchitectural studies. Together, these results suggest insular lateralization in processing different aspects of taste stimuli in men.

### Acknowledgements

This research was funded by Top Institute Food and Nutrition (TIFN). Furthermore, the authors would like to acknowledge Luca Nanetti for designing the gustometer, for help with pilot testing and the experimental design.

